II. *Transmission of Flagellates Living in the Blood of certain Freshwater Fishes.*

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(*Communicated by* Dr. C. J. MARTIN, *F.R.S.*)

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(From the Department of Protozoology, Lister Institute.)

[PLATES 1 AND 2.]

THE work recorded in this paper deals with the transmission and life-history of certain trypanosomes and a trypanoplasm, inhabiting the blood of freshwater fish. It was undertaken at the suggestion of Prof. MINCHIN, to whom I am indebted for much kind advice.

In 1905, Dr. PETRIE (15) had observed that the goldfish in the pond at Queensberry Lodge, in the garden of the Lister Institute at Elstree, were almost invariably infected with a trypanosome. Three years later, Dr. J. D. THOMSON (20) again found the flagellate in question, and gave an account of the appearances presented in cultures grown upon blood-agar. The trypanosome infection still persists in the blood of the fish in this pond, and, in addition, a trypanoplasm has appeared since 1908, when Dr. THOMSON carried out his work.

It was obvious that the problem of the transmission of the flagellates presenting itself in so restricted an area was a particularly favourable subject of study. The pond is quite isolated and is fed by surface drainage. Wild duck visit it occasionally.

During the early part of the summer the pond was full of weed belonging to the genus Potamogeton. On dredging with a net, Nepa, Notonecta, Corixa, various hydrometrids, and Dytiscus were found, as well as a number of small larval forms; none of these were found to play any part in the transmission of the fish-parasites. Several specimens of Argulus from a pond at Histon, in Cambridgeshire, where many of the fish showed trypanoplasms and trypanosomes, were examined; they contained, however, no protozoan parasites and did not appear to feed upon blood. I never found Argulus in any of the Elstree ponds.

Nepa cinerea from the Queensberry Lodge pond showed infection with a long slender Crithidia, presenting quite a different appearance from the Herpetomonas (*H. jaculum*) described by LÉGER (9) and by Miss PORTER (18). The form described by these authors was subsequently obtained in Nepa sent me by Messrs. BOLTON,

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of Birmingham, so that the two parasites could be compared with each other. The origin of the Crithidia in Nepa is not clear; it may be a natural flagellate of the Nepa, or possibly derived from one or other of the animals it preys upon. I found that Nepa will attack small leeches. This part of the work was, however, not carried far enough to warrant any conclusions being drawn.

Finally, a number of leeches belonging to the species *Hemiclepsis marginata* were found, and these proved to be infected with both trypanosomes and trypanoplasms. Curiously enough, no other leeches were met with in this pond, except a single specimen of *Glossosiphonia complanata*, which showed an infection with a trypanoplasm. This leech is said to feed exclusively on molluscs, aquatic annelids, and small insect-larvæ; I have not so far succeeded in getting it to feed on fish. It is, on the whole, very probable that the trypanoplasm of *G. complanata* is derived from one or other of the molluscs preyed upon. A parasite of this type has been described from the common Helix by FRIEDRICH (3). That author seems, however, to be of the opinion that the whole life-cycle of the flagellate takes place in the mollusc. BRUMPT also mentions the occurrence of trypanoplasms in *G. complanata*, and is inclined to consider that they are transmitted from parent to offspring (1).

Hemiclepsis marginata from the Queensberry pond attacked the goldfish very readily. Fasting individuals were put on clean (*i.e.* non-infected) fish, with the result that, in one case, a very good trypanoplasm-infection appeared, while in the other case a slight infection with both trypanosomes and trypanoplasms was produced. This gave a basis for more precise experiments.

In order to obtain really convincing evidence in work of this type, the requisite factors are clean Vertebrates and clean specimens of the transmitting agent. As regards the fish, circumstances were particularly favourable. Mr. T. H. RICHES very kindly placed goldfish from an artificial pond in his garden at my disposal. These creatures had been very carefully examined some years ago by Mr. RICHES, Prof. MINCHIN, and Dr. THOMSON, and found to contain no parasites; they still showed no flagellates when investigated this summer. It is practically impossible to obtain a reliable source of clean specimens in nature, more especially when, as in fish, the infections are of a slight and chronic type. I found, as a matter of practice, that, as will be seen later, clean leeches could be used to test the blood of fish which do not show parasites in the course of ordinary microscopic examination. If a batch of 5–10 small, clean leeches have fed on the blood of the fish in question, and do not any of them, when dissected, show flagellates, it may be assumed that the fish is not infected.

The supply of clean leeches was obtained as follows. About half a mile from the Queensberry Lodge pond there is a reservoir which supplies the Grand Junction Canal. It is well stocked with fish, bream, pike, perch, rudd and roach being all fairly abundant. Wild duck and water-hens come in good numbers and nest in the reeds at the water's edge. These reservoir fish very generally show trypanosomes

and trypanoplasms in their blood. Upon hunting carefully among the broad-leafed rushes, I found that leeches of almost every British species were to be obtained in good numbers. The following is a list of those collected :—

(1) *Helobdella stagnalis.*	(5) *Herpobdella octoculata.*
(2) *Glossosiphonia heteroclita.*	(6) *Hæmopis sanguisuga.*
(3) *Glossosiphonia complanata.*	(7) *Protoclepsis tessellata.*
(4) *Herpobdella atomaria.*	(8) *Hemiclepsis marginata.*

A few remarks upon the habits of these leeches are necessary to make clear the conditions obtaining in the reservoir. The facts recorded are derived partly from personal observation and partly from Mr. W. A. HARDING'S excellent account of British leeches (5).

(1) *Helobdella stagnalis* is a very common form in the reservoir and also in the neighbouring ponds. It broods its eggs and feeds upon the juices of Gasteropods, and also upon the larvæ of Chironomus. I have observed that it attacks the embryos of quite young leeches, still showing yolk, of the genus Hemiclepsis, in cases where these have been detached from their parents. Flagellates were never found in Helobdella, but a ciliate parasite belonging to the genus *Anoplophrya*, and closely allied to the species *A. paranaidis*, PIERANTONI (16), parasitic in the alimentary tract of a Paranais from the Gulf of Naples, was found pretty frequently in these leeches, both in specimens from Elstree and also from Histon, in Cambridgeshire.

(2) *Glossosiphonia heteroclita* broods its eggs and feeds upon the juices of Gasteropods. Anoplophrya is the only Protozoan parasite found by me in this leech, which is a common species in the reservoir and in the Elstree ponds generally.

(3) *Glossosiphonia complanata* is chiefly parasitic on Limnæa, Planorbis, and other freshwater molluscs. It lays its eggs (according to various authorities) on weed or some foreign body and broods them. Protozoan parasites were not observed, except in the one individual already mentioned from the Queensberry pond, where trypanoplasms were found in the proboscis and anterior part of the crop. It may be observed in passing that these trypanoplasms were examined in tap-water and were actively motile, while the trypanoplasm found in the proboscis-sheath of Hemiclepsis died when set free in tap-water. This leech was fairly common in the reservoir, but was not found in any of the neighbouring ponds, with the exception of the single specimen cited above.

(4) and (5) *Herpobdella atomaria* and *H. octoculata.* These leeches are very much alike in appearance and are closely allied species. They lay their eggs in transparent capsules on water-weed. They chiefly feed upon small Oligochætes. I found that these two species were present in large numbers in the reservoir, but not elsewhere in the other ponds investigated. *Orcheobius herpobdellæ*, a Coccidian described by SCHUBERG and KUNZE (19) was obtained from these forms in specimens from Histon and Elstree.

(6) *Hæmopis sanguisuga* was also found in the reservoir, but not in large numbers. This leech is not a blood-sucker, notwithstanding its bloodthirsty title. It is a carnivorous creature of catholic tastes, devouring worms, several species of leeches, tadpoles, molluscs, and insect-larvæ; it is even said to attack small fish and newts. Protozoan parasites were never observed in this leech.

(7) *Protoclepsis tessellata* is a true blood-sucker. It is a very active leech and is said to feed upon the blood of wild-fowl. A number of newly-fed specimens were examined and found to contain nucleated blood-corpuscles smaller in size than those found in fishes or amphibians, and most probably derived from the numerous wild duck which live upon the reservoir. This leech is said to be very rare in England; it is, however, abundant in the Elstree reservoir. I never found it in other places. It did not show any protozoan parasites.

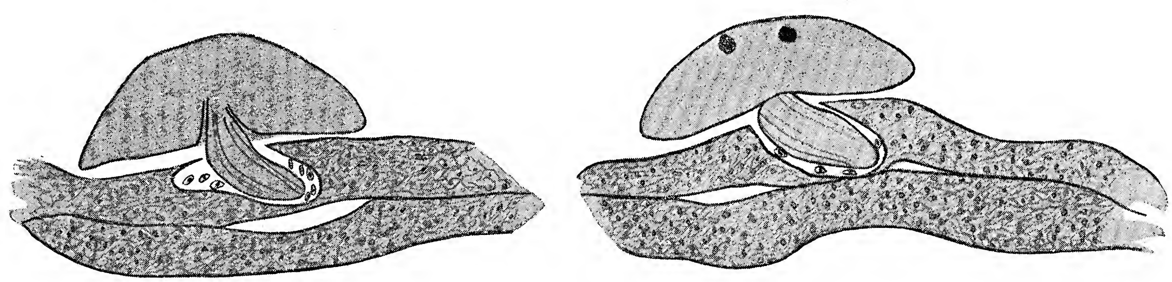
(8) *Hemiclepsis marginata*, a common form in the Elstree reservoir, is also a true blood-sucker and attacks fish. I have seen it feed on goldfish, perch, bream, roach, rudd, tench, pike and eels, and have no doubt that it would probably attack many more species. Hemiclepsis, like Protoclepsis, is said to be of rare occurrence, but is, I expect, a much more common parasite than has hitherto been supposed. It is a difficult leech to find, but from my own experience and that of Mr. HARDING, I am much inclined to think that it has been overlooked. This leech has of course a number of natural enemies. I found that Nepa and Dytiscus will attack pretty big specimens. *Trocheta subviridis*, a large semi-terrestrial leech, devours them eagerly; fish also attack them very readily, but never become infected with trypanosomes by this channel. The young specimens of Hemiclepsis are, I have noticed, preyed upon by insect-larvæ and by leeches of the species Helobdella. I have no doubt that many other creatures may attack Hemiclepsis, but these are the only cases I happen to have observed.

During the summer and autumn months I found in the reservoir a number of specimens of Hemiclepsis with broods. These individuals were isolated in beakers with some water-weed, and the young grew up successfully in the majority of cases. The weed is rather important, as the leeches seem to thrive much better when it is present. The water in the beakers need only be changed very occasionally. It was noticed that eggs deserted by the mother died, but quite young embryos not infrequently developed by themselves. If a leech is harassed it usually deserts its brood. The young creatures have at first a good deal of yolk, which may be of a brilliant apple-green, a bright yellow-green, or an opaque white colour. By the time the yolk is absorbed the young leeches usually begin to desert their mother, and are ready for their first feed of blood. They can, however, persist for months without any food at all.

Some individuals from each brood were allowed to feed on clean fish. The small animals take hold of their host very readily; they force their way in between the scales and seem to have no difficulty in piercing the skin. A favourite method of

attack is for the leech to penetrate into the external nares. During the act of sucking the proboscis is extruded and pierces the tissues of the fish (see text-fig. 1), while slow and more or less rhythmic contractions pass backwards along the body of the leech; these are more marked during the earlier stages of feeding. Hemiclepsis does not possess jaws or teeth.

More than 70 young leeches from 12 different broods were fed on clean goldfish, and none of them developed flagellates. With the exception of four individuals, which were examined before they had fed, all these leeches were allowed to remain for a varying number of days after their first feed, in order to permit the flagellates, if any were present, to develop and multiply; some specimens were kept till after their second clean feed. Neither trypanosomes nor trypanoplasms ever appeared in any of these control leeches. This result, taken in conjunction with the precisely similar experience of BRUMPT (1), who carried out analogous experiments with over a hundred young individuals of this species, seems to establish the fact that, in the case of *Hemiclepsis marginata*, trypanosomes and trypanoplasms are not transmitted



TEXT-FIG. 1.—Two Consecutive Sections of the Head of a Leech in the act of Sucking Blood from the Tail of a Goldfish.

from parent to offspring. It may be mentioned in passing that every "wild" Hemiclepsis hitherto examined has shown a greater or less infection with either trypanoplasms or trypanosomes or both.

Having established that the young leeches were clean, it remained to use them as the vehicle of transmission and to observe the features of the process.

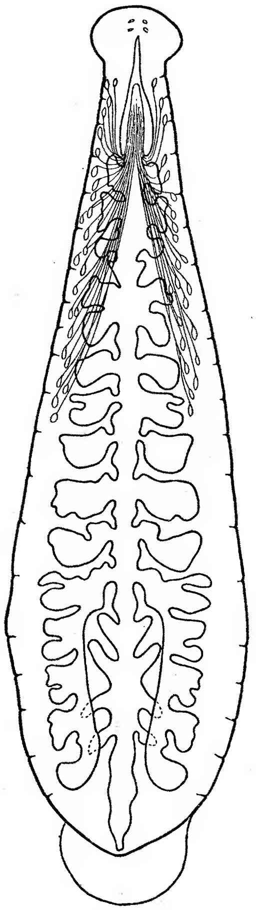
Anatomy of the leech Hemiclepsis.

It is necessary to give a brief account of certain points in the anatomy of the leech, more especially those concerning the alimentary tract (see text-fig. 2).

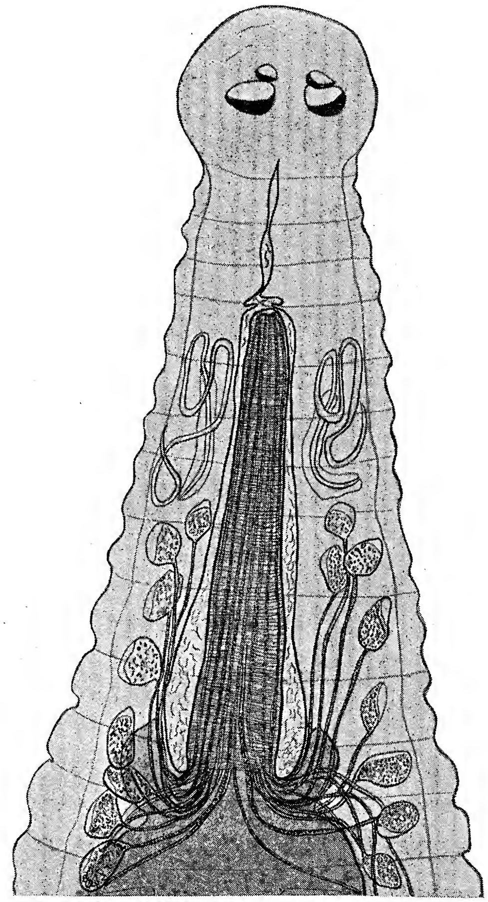
The mouth lies roughly in the centre of the anterior sucker and leads into a somewhat thin-walled collapsible sac, in the centre of which is the muscular proboscis (see text-fig. 3). This thin-walled sac is the proboscis-sheath and ends blindly at the posterior end, where it becomes confluent with the proboscis. The latter, which can be extruded at will, leads into the large lobed crop. From the crop there opens the stomach, with four diverticula on either side, and from the stomach arises the intestine, a simple coiled tube leading to the exterior by means of the short straight

rectum. Just at the base of the proboscis, at the point where it joins with the crop, there enter the many ducts of the salivary glands, which run forward in the wall of the proboscis and open at its extremity.

The salivary gland is not a compact organ but is simply a number ot single-celled elements, each with its own duct; they are very numerous and stretch through a considerable part of the length of the body. They secrete a fluid containing many bright refractile particles, which in the live specimen can be seen to pass down the



TEXT-FIG. 2.—Diagram of Alimentary Tract of *Hemiclepsis marginata*. (After W. A. HARDING, slightly modified.)*



TEXT-FIG. 3.—Anterior end of *H. marginata*, drawn from transparent living specimen. The flagellates may be seen as very minute threads in the proboscis-sheath.

ducts to the tip of the proboscis (see text-fig. 3). The proboscis is only extruded while the leech is feeding, and in the long period during which it is retracted the salivary fluid pours out into the sheath surrounding it; it is thus always more or less bathed in the secretion from the glands.

The above description of the relations of the salivary glands applies also in the case of the other British fish-leech, Piscicola. I may remark that the points noted above are very clearly visible in living specimens investigated under the lower powers of

* I am indebted to the editors of 'Parasitology' for permission to use this figure.

the microscope. I have not seen any account of these glands which mentions the prolongation of the ducts down the proboscis. Little attention has, however, been paid to the anatomy of the species in question.

Transmission of Trypanoplasms.

In the following experiments dealing with trypanoplasms, only those found in the Queensberry Lodge goldfish were used. It is highly probable that this trypanoplasm in the goldfish is *Trypanoplasma cyprini* of PLEHN (17). The relative positions of the trophonucleus and kinetonucleus correspond to those shown in the figure given by LÜHE (11). It also resembles very closely *T. keysselitzi*, found in the tench by Prof. MINCHIN (12), who considers that *T. keysselitzi* may prove to be a synonym of *T. cyprini.*

A pure trypanoplasm infection was brought about by chance, by allowing two Hemiclepsis from the pond to feed upon a clean goldfish. On the 4th day after inoculation* by these leeches, slender and rather small trypanoplasms appeared in the blood. On the 6th day the flagellates were more numerous, and some had increased in size. By the 14th day the slender small forms were rare, and the majority of the creatures had assumed the large size, and somewhat euglenoid movement characteristic of the normal blood-dwelling type (Plate 1, fig. 1). Young clean leeches were allowed to feed on the blood of this fish on the 23rd day after inoculation. They fed from 3.30 P.M. to 10.30 P.M. One leech, which had only taken a very small quantity of blood, and was therefore very transparent, was examined alive under a cover-slip. The trypanoplasms could be seen moving in the sacs of the crop through the body wall, and at 9 o'clock, *i.e.*, about 4–5 hours after beginning to feed, the flagellates were to be seen dividing. When first taken into the crop of the leech, the trypanoplasms become rather broader and bulkier in appearance, and show a very characteristic flowing kind of motion (Plate 1, figs. 2 and 3). After the first few hours they are frequently to be seen in division; the last stage of this process is passed through with great rapidity. On the 2nd day after feeding, slender forms, somewhat comma-shaped, appear in small numbers; they are much less undulating in their movements than the broader forms. These two types (Plate 1, figs. 4–5*a*) persist side by side for some time, the slender forms gradually increasing in number; intermediate forms are also present. After some days the slender forms move forward into the proboscis-sheath. They may occupy this situation as early as the 6th day after feeding. As time goes on they come to lie there in incredible numbers, and very generally attach themselves to the wall by their anterior end, *i.e.*, the end with the free flagellum (Plate 1, fig. 6). In young leeches the flagellates in the proboscis-region can be seen with perfect clearness through the wall of the living specimen, and thus the condition of the

* The word "inoculation" in this paper always implies the agency of leeches. Inoculation by artificial means was never practised.

leech in respect to its infectivity may be readily ascertained. It is quite clear that the trypanoplasms arrive in the sheath by the simple method of coming up the lumen of the proboscis and out into the surrounding space. They can be seen in the act of doing so in suitable living specimens.

While the trypanoplasms are to be found all through the crop, there is a distinct tendency for them to assemble, towards the end of digestion, in the anterior part immediately behind the proboscis. This is particularly marked in the case of infected leeches which have been given a feed of clean blood. It is difficult to understand the very early appearance of the trypanoplasm in the proboscis-sheath, as the leech, even when quite small, is never ready to feed again for 10 days, and generally a much longer time must elapse before it is ready for another meal. The time between successive feeds is determined by the size of the leech, the amount of blood ingested, and the temperature. Leeches can sometimes be induced to feed before they have completely digested the previous meal, but they do not take hold readily, and only take little blood.

On the 11th day after feeding, three leeches were put on a clean fish (I), and fed for about $5\frac{1}{2}$ hours. This fish (I) showed no flagellates when examined on the 4th day after inoculation, but rare flagellates were seen in the blood on the 7th day.

It is interesting to find out how the protozoan parasite behaves when the invertebrate host is fed on clean blood. At end of digestion, a leech infected with trypanoplasms shows immense crowds of the parasite in the proboscis-sheath, a considerable number in the anterior part of the crop, and practically no specimens further back. In the case of trypanoplasms, a single clean feed may sometimes clear the whole leech of flagellates; this, however, appears to be exceptional. Generally speaking, one clean feed does not immediately clear either the proboscis-sheath or the crop. Sometimes the sheath is quite crammed again with the flagellates within 24 hours after a clean feed. I have observed this in the leech Piscicola, as well as in Hemiclepsis. The trypanoplasms are of the true proboscis type. The explanation is that they are probably the remaining individuals in this condition which were in the crop, and which have come forward into the proboscis-sheath when the new blood was ingested. I have not infrequently found that, when the uninfected blood is digested, all the trypanoplasms are aggregated in the proboscis-sheath, and none at all are to be found anywhere in the crop.

Experiments are still going on to determine the effect of a second clean feed, and also to discover how long trypanoplasms will persist in the proboscis of the fasting leech.

Transmission of Fish-trypanosomes.

The question of the transmission of fish-trypanosomes was found to be a much more complex problem, as I was here working with a number of so-called different species. I found that trypanosomes derived from goldfish in the Queensberry Lodge

pond, and from bream and perch out of the Elstree reservoir, and from rudd from Histon, would all complete their cycle in clean Hemiclepsis. I use the word "cycle" to denote the very regular and marked succession of stages which the trypanosome passes through in the crop of the leech, and which culminate in the definite slender inoculative type found in the proboscis-sheath.

Not only did the trypanosomes from these different sources invariably succeed in arriving in due course of time in the proboscis-sheath of the Hemiclepsis, but, in the case of the goldfish, bream, and perch, they could be transmitted to clean goldfish by allowing the leeches to feed upon them.* I am not yet in a position to state that the trypanosomes from these different sources belong in reality to one species; further experiments must be carried out before any definite opinion can be arrived at. Nevertheless, the evidence at present available is tending towards a reduction in the number of species.

So far as can be judged from observations on the living organisms, the cycle gone through in the leech appears to be identical in the case of each of the trypanosomes mentioned. This description is drawn from observations on newly-hatched leeches receiving their first feed.

The trypanosomes are taken into the crop of the Hemiclepsis along with the blood. About six to nine hours after being ingested the flagellates begin to divide; in cold weather this time is somewhat increased. The division is of a very characteristic type, and consists in the budding off of a small, broad Herpetomonas-like form from the posterior end of the original trypanosome, which remains motile throughout the process. The daughter-individual presently develops an undulating membrane, assumes a somewhat broad crithidial condition, and proceeds to divide in turn after a few hours. The details of the first division will be gone into more fully later on in the paper (see Plate 1, figs. 7, 8, and 9, and text-fig. 4, p. 44); it suffices, for the present, to say that both parent and daughter-individuals go on dividing, so that, in two to three days, the crop is peopled with numbers of broad, somewhat spear-headed or tadpole-shaped flagellates, with the kinetonucleus anterior, or just posterior, to the trophonucleus (figs. 10–13). That is to say, they are in a crithidial condition as regards their nuclei, but the body is much broader in shape.

Multiplication proceeds, and it must be borne in mind that there is a very striking amount of variation in the method of division in respect to the protoplasmic body of the parasite, and a general tendency to unequal fission. I have over and over again spent hours continuously watching specimens of unusual appearance in the hope that they might be conjugating individuals, and have in every case found that division and not fusion was in progress. The multiplication that goes on during this period is positively amazing, and the crop becomes filled with the flagellates even when only

* At the time of writing the trypanosome of the rudd has not as yet been passed into clean goldfish, as the leeches have not yet been allowed to feed.

a very few have been originally ingested. Presently some of the individuals begin to lengthen out, and there is a general increase in size (figs. 12, 14, and 15). As a rule this does not take place until after the fifth to seventh day; from the eighth day onwards very slender elongated trypaniform individuals arise by division from the broader long forms (Plate 2, fig. 16). These very slender creatures are the inoculative type (figs. 17 and 18); they appear at first in quite small numbers, but predominate greatly as time goes on, and end by being almost but never quite exclusively the only form present. The final stage arises quite gradually from the broader creatures, and every intermediate stage is for a time represented in large numbers.

From about the 10th day onwards these very slender trypanosomes continue to pass forward into the proboscis in increasing numbers, and are to be seen lying in the sheath. They do not attach themselves to the wall in the same regular way as the trypanoplasms. They can be seen moving actively in the sheath, but have a tendency to get crowded together at the posterior end. Trypanosomes may appear in the sheath as early as the ninth day, and in one experiment three leeches feeding on a clean goldfish on the 10th day after an infected feed, produced an infection in the clean individual. This is, however, unusually early; the leeches were quite newly hatched, and the weather was warm.

I wish to emphasise particularly that the time-factor in the whole of this development is subject to great variation; trypanosomes may not appear in the proboscis-sheath for more than 30 to 35 days or even longer. (This refers to young leeches having their first feed.) The point is one which seems to depend entirely on the rapidity of digestion of the leech.

The appearance of the inoculative type and its migration into the proboscis are, so far as I can see, the response to some chemical or physical stimulus. If the leech is larger the digestion goes on much more slowly, and the middle period, during which there is a great range of form in the trypanosomes, is of very long duration (several months), and the appearance of the inoculative type in the proboscis-sheath is correspondingly delayed. The sheath contains no trypanosomes as a general rule until some time after the leech has absorbed all the red blood in its crop. One may often find the crop crammed with slender forms of the inoculative type, or in stages nearly approximating to it, while the sheath is still quite clear from flagellates. The trypanosomes get much more firmly established throughout the crop than is the case with the trypanoplasm; it is no infrequent occurrence to find the whole crop simply seething with amazing numbers of the creatures. I have never found the stomach or intestine to be infested with them.

The leech is not infective until the slender forms are in the sheath, even though the rest of the leech is full of trypanosomes. Occasionally the leech is willing to feed before the flagellates reach the proboscis, but this seems to be unusual in the young individuals; it is not so rare in adult leeches. I have not up to the present

observed division in the inoculative type. If a leech is not allowed to feed when digestion is quite complete and the sheath is full of trypanosomes, there seems to occur a certain amount of degeneration and death among the parasites, but new forms keep coming up from the crop to supply the deficiency. In a young leech, after its first feed, I have found trypanosomes to persist in the sheath for more than 61 days from the time of their first appearance in that situation. Of course in nature a leech may very probably have to wait a long time before it happens to find a fish, and the very large numbers of flagellates produced seem to be adapted to meet this circumstance.

A rather interesting point in reference to the digestion of the leech was observed accidentally. Some clean leeches, which had fed on the blood of an infected pike, were put into an incubator at a temperature of 25° C. in order to hasten the digestion of the meal. Another batch of leeches fed on the same fish were left at room-temperature. The leeches in the incubator seemed quite happy and digestion proceeded more rapidly. After about ten days some of these leeches were examined, but to my surprise contained no parasites at all. I then opened one of the specimens which had not been incubated, and found it to be literally crammed with trypanosomes. At the same time I had put into the incubator two leeches containing perch-trypanosomes; in these specimens the red blood was all digested before they were put into the incubator. The specimens were very transparent, and I had observed very numerous flagellates in the crop through the body-wall before putting them into the incubator. My purpose in subjecting them to the greater heat was to hasten the appearance of the inoculative form in the proboscis-sheath.

In these specimens the trypanosomes remained alive, therefore their disappearance in the other case was not a question of absolute temperature. The explanation seems to be that, at the higher temperature, the leech is able, during the active period of secretion, simply to digest the trypanosomes. The same thing occurred with some leeches containing rudd-trypanosomes, but in this case I had unfortunately put the whole batch into the incubator.

It may be mentioned in passing that leeches sometimes suck lymph instead of blood. The trypanosomes in an infected fish seem to be as numerous in the lymph as in the blood. This circumstance is rather fortunate, as young leeches which have fed on lymph remain very transparent.

The effect of a single clean feed on a leech infected with trypanosomes differs considerably from that produced in the case of an infection with trypanoplasms. The proboscis-sheath in a trypanosome-infection is invariably cleaned entirely of the flagellates, but I have never up to the present found that a single clean feed causes the parasites to disappear from the whole leech.

It is a point of some interest to determine which type of trypanosome carries on the infection in the intermediate host. The forms found in the crop of infected leeches immediately after a clean feed are rather small, broad, trypaniform and

crithidial creatures (see Plate 2, figs. 19–21); sometimes they are of a curious squat type with a very broad undulating membrane. Rare proboscis-forms, very slender, may also be present, but these usually show quite clear signs of degeneration.

After 36–72 hours the trypanosomes show the stages typical of the early period of digestion in the leech, and it is quite impossible to tell by inspection whether the infection is a primary one, derived directly from an infected fish, or a secondary infection dating from the last feed but one (see Plate 2, figs. 23–27). Numerically, these secondary infections are soon just as heavy as a good primary infection, and the inoculative type of trypanosome does not appear in the proboscis-sheath any sooner than in a primary infection, that is to say, not until some time after all the red blood has been digested.

It is a point of some importance how closely adapted the inoculative form of these trypanosomes is to the condition obtaining at the end of digestion. Although the strain of trypanosomes as a whole is not dislodged by feeding on clean blood (certainly not by one such feed, and probably not by several), nevertheless the inoculative type, as such, comes and goes in direct correlation with the digestive phases of the leech, and probably in response to some quite definite environmental stimulus.

The forms from which these secondary infections originate are certainly in part derived from the ever-present residue of broad and sometimes nearly spherical individuals, which remain when the large majority develop into the inoculative type. I have not as yet been able to determine quite definitely if they are the only source. It is important to discover if any of the slender proboscis-forms are capable of reverting to the broad phase. Up to the present the evidence is against this, and degeneration seems to overtake forms of this kind left over in the crop from a previous meal. Careful observation of larger numbers of leeches at this stage will, I hope, make this point perfectly clear.

If an infected leech is given a feed of blood containing trypanosomes of the same kind, *i.e.*, derived originally from the same species of fish (the bream was used for one experiment), the newly-ingested flagellates can only be distinguished from the forms already there for about 24–36 hours, that is to say, only so long as they still retain certain features characteristic of the parasite in the blood of the fish. Conjugation was most carefully watched for in the leeches of this last experiment, as it was hoped that the persisting trypanosomes might fuse with the newly-ingested forms. This expectation was, however, not fulfilled. Up to the present all attempts to find the moment of conjugation in these flagellates have been unsuccessful. This does not necessarily invalidate the theoretical expectation that it must occur at some period.

It must be borne in mind that, particularly in flagellates, conjugation rarely occurs at regular intervals. The idea that it must take place once in every cycle, *i.e.*, in every passage through the intermediate host, is an unconscious analogy drawn from

the life-cycle of the malarial parasite or from that of the Coccidia. It is natural enough that workers should be a little apt to think in terms of the most distinctive and best-known life-histories. I think, however, that in the trypanosome question this has somewhat confused the issue. It is more helpful to draw upon the few well studied flagellate histories for general considerations concerning difficult and obscure points in the form under discussion.

As trypanosomes seem generally to become established for life in the intermediate host, conjugation might occur in one out of quite an indefinite number of cycles. For instance, starting with a young leech, conjugation might occur at some point in, say, the 5th cycle, and not perhaps again for quite a number of feeds.

If trypanosomes and trypanoplasms are both present in one leech they each run their characteristic cycles. Generally one or other of the two predominates. A leech may, however, infect a clean fish with both parasites at one time. Up to the present the following passages were effected by means of clean young leeches:—Goldfish to goldfish, perch to goldfish, bream to goldfish. Goldfish to clean eel, and bream to clean eel, were both attempted, so far without success, but the experiment is still in progress. An experiment is under way to see if pike-trypanosomes from another district can be passed into clean goldfish. At the present time the trypanosomes are developing along the usual lines, but have not yet reached the inoculative stage.

It was found that "wild" trypanosomes from adult leeches in the reservoir appeared in the blood of goldfish (clean) upon which they had fed. I have no information as to what species these trypanosomes belong to, but should suspect that they were derived from either perch or bream.

Behaviour of the Trypanosomes in the Fish.

Generally speaking, trypanosome-infections in fish are of a slight and chronic type, the number of parasites in the blood being relatively very low. In my experience small-sized perch have shown the best natural infections. The fish do not, as a rule, show any pathogenic symptoms, though occasionally there is quite a marked anæmia; the gills become exceedingly pale in colour, and the blood is watery, and seems reduced in quantity relatively to the size of the fish. Occasional deaths occur, but it is difficult to be sure that the trypanosome is the cause.

I have obtained no information whatsoever as to how the parasite multiplies within the vertebrate host, having never come across division-stages. There is often a great variation in size in the trypanosomes of one infection, and an increase in the number of parasites seems to synchronise with the appearance of small forms, but I am quite in the dark as regards their origin. These occasional exacerbations of the infection are never very marked, and are of rare occurrence; I have not come across them sufficiently often to have obtained a really satisfactory insight into their nature.

The usual course of an infection is as follows. (It must be mentioned that the clean fish were infected in the late autumn, and have not been under observation for more than a few months. I do not know if the season of the year exercises any influence on the course of the infection, but should not expect *a priori* this to be an important factor.) If leeches in the right condition are allowed to feed on a clean goldfish, trypanosomes may be found by ordinary microscopic examination as early as the 5th day; they may not appear, however, till much later. The early finding of trypanosomes in the usual way by direct examination depends, of course, on the number of individuals injected by the leech, as well as on the subsequent multiplication in the fish. The flagellates which appear early are of a type quite markedly smaller and more slender than those seen at a later period. The time elapsing before the appearance of the normal form varies within a few days. Although I have never seen trypanosomes in live films before the 5th day, nevertheless, a fish may already be infective for young clean leeches as early as 48 hours after the infected ones have fed. This time is also subject to variation.

It is interesting from the point of view of the biology of the trypanosomes to find that they can resume the active multiplicative condition once more after so short a sojourn in the blood of the fish. Increase in the numbers of the newly injected parasites occurs in the blood during the early days of the infection, but this soon ceases, and the numbers become stationary. Much attention cannot be paid to slight variation in the numbers of trypanosomes counted in a film at the different examinations, as the question of chance enters to some extent. Often there is a gradual decrease, and periods when no flagellates appear in the blood supervene; the blood is, nevertheless, infective to clean leeches. Slight recrudescences occur from time to time.

One fish which showed a slight infection with both parasites has been under observation for some months. The blood is still capable of producing trypanoplasm-infections in clean leeches, but has ceased to produce trypanosome-infections. This creature is still under observation, and it will be interesting to see if the trypanosome will appear again at a later date. One infection, even when it has become so slight as to be apparently latent, does not protect against a subsequent infection from a second leech. This was shown by the following experiment:—A rather large goldfish (Fish 22) from the Queensberry Lodge pond showed a slight natural infection with trypanosomes. After being in captivity for some time, flagellates were no longer to be found in the blood. Two "wild" Hemiclepsis from the reservoir were allowed to feed on this fish, and 10 days later a number of trypanosomes were observed in its blood, as many as eight being found in one wet film under a $\frac{3}{4}$ inch by $\frac{3}{4}$ inch coverslip. The numbers decreased again rather rapidly, and on the 20th day after the leeches had fed only one trypanosome was found in a film of blood. This point, however, needs further elaboration, as it would be of interest to see if a fish would reinfect with its own strain of trypanosomes, that is to say, by a leech whose parasites were derived from that same fish's blood while still infective.

To turn to the question of the different species of trypanosome, I find that the cycles in the leech are indistinguishable; the morphological features in the fishes show only slight differences, which in so labile a form as a trypanosome gives a very unsure basis for specific distinctions. The criticism of the data at my disposal is that while the observations made on the leech have been carried out on relatively large numbers of individuals, those upon the fish have been made upon relatively very small numbers of animals.

Further experiments, and particularly those dealing with the passage of trypanosomes derived from one "wild" fish into clean specimens of another kind of "wild" fish, are requisite before the matter can be settled.

The origin of the trypanosomes which appeared in the Queensberry Lodge pond can, of course, never be definitely settled. I am strongly inclined to think that they must be derived from the reservoir, probably by leeches brought by duck, and that they are therefore one of the species found in the reservoir-fish.

It is interesting to observe how much simpler the life-cycle of the trypanoplasm is than that of the trypanosome, both as regards the morphological feature of the protozoon and as regards the precision of its adaptation to the condition in the leech. The changes of form in the case of the trypanoplasm are quite trifling, whereas those in the trypanosome are so considerable that for long periods these parasites might be considered to belong to a different genus, namely, Crithidia. Further, the trypanoplasm does not show the very precise correlation between the phases of the parasite and the state of digestion in the leech. There is absolutely no evidence to suggest even a remote phylogenetic connection between the two forms.

Effect of Reagents on the Trypanosome in the Blood of the Fish.

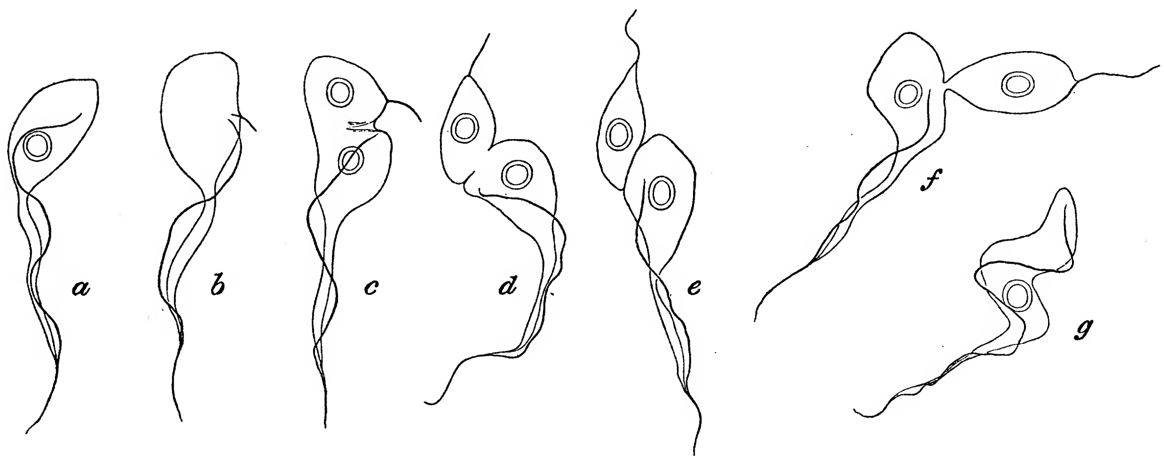
In the early part of the investigation attempts were made to see if there was any likelihood of direct infection occurring from fish to fish, and in this connection experiments were made to see how the trypanosome reacted to the presence of water. I may say at once that no direct infection takes place. The trypanosome, nevertheless, reacts in a very characteristic way to the presence of water. If a drop of blood from a fish showing a normal infection is mounted on a slide with an approximately equal amount of either tap-water or distilled water, the trypanosomes undergo a number of changes which culminate in division.

To quote an individual experiment,* a slide was prepared with tap-water as described above at 3.45 P.M. on August 4. At 9.45 a trypanosome was selected for observation, and was watched continuously till 2.50 P.M. on August 5. This creature already showed the first alterations, that is to say, the body had become very much broader at its non-flagellate end. Individuals at this stage are very often in the

* I am indebted to Dr. HENDERSON SMITH, of the Lister Institute, for kindly relieving me at the microscope from time to time, so that these observations could be carried out continuously.

shape of a dumpy spiral (see text-fig. 4, *g*). The whole creature had become somewhat shorter, but the anterior (flagellate) end tapered out in the characteristic way. The posterior end became still more thickened and presently somewhat club-shaped, and the flagellum no longer had its origin at the posterior extremity of the body, but arose now from a point considerably further forward. The trophonucleus was very clearly visible just at this time; after a little while it disappeared, and presently, about twenty minutes later, two nuclei were to be distinguished.

There now grew out from a point quite close to the origin of the flagellum a little stiff process which gradually lengthened out and became motile; this was the flagellum of the daughter-individual. A constriction began to appear in the club-shaped thickened end, and there was gradually split off an actively motile pear-shaped creature. It had as yet no undulating membrane, the flagellum striking straight out as in a Herpetomonas. In many cases, however, the creature had already



TEXT-FIG. 4 (*a*—*f*).—Sketches of one living Trypanosome, showing different moments in the process of division. × 2000. The trypanosome is from the blood of a goldfish, and is dividing in response to the admixture of water with the blood. This method of division is identical with the first divisions in the crop of the leech; *g* shows the short spiral shape which is usually the first reaction to the presence of the water.

approached the crithidial phase by the time it was set free. The parent had never ceased to move all through this process, and preserved its original locomotor apparatus intact. The young animal is simply budded off from the thickened end.

In the trypanosome under observation the daughter form had got completely free by 1.25 A.M. on August 5. The parent and daughter remained in the same field, and were kept under observation. The former was still somewhat broad and club-shaped, and did not resume the elongated condition found in the undiluted blood. By 5 A.M. the nucleus in the parent disappeared; by 5.20 a second flagellum had grown out, and at 6.50 A.M. this second daughter-individual was thrown off. During this same period the first daughter, which had now begun to develop an undulating membrane, divided also, but did so by longitudinal fission. At the

beginning of this division it appeared as though the grand-daughter would be smaller than the daughter, but the inequality disappeared, and the two creatures were practically of the same size when separation took place.

The original parent had thus split off daughter (I) at 1.25 A.M., and had then split off daughter (II) at 6.50 A.M.; daughter (I) had in turn given rise to grand-daughter (I), the separation being complete at 7.30 A.M. At 11 A.M. daughter (II) began to divide, and at 11.5 the parent started to divide for the third time, that is to say, to throw off daughter (III). Grand-daughter (I) began to divide at 1.50 P.M. Unfortunately, at 2.50 P.M. on August 5, the trypanosomes ceased to move, their death being probably due to the development of bacteria on the slide.

To put the result of the foregoing observations in brief, the trypanosome, under the condition of dilution of the blood given above, divides after about six to nine hours, and the products of division in turn divide after a similar period, a third division occurring again in six to nine hours. These observations have been repeated over and over again as far as the first division, and somewhat less frequently to the second. The time-factor in these divisions varies somewhat, especially in relation to temperature.

It has been observed that sometimes a few individual trypanosomes react very tardily, or not at all, to the stimulus of the water, and occasionally a whole infection is found where none of them react. These are generally, so far as my observation goes, very slight infections, and the flagellates are very slender. The exact bearing of this is not yet clear. Distilled water which has been boiled and allowed to cool is just as effective as tap-water in producing the division. In blood quite unmixed with water no multiplication occurs, nor in blood mixed with 1 per cent. salt solution.

The trypanosomes will live for a couple of days and remain quite active and unaltered without multiplying on a slide of blood mixed with 1 per cent. potassium chloride, likewise in 1 per cent. ammonium nitrate. This last was tried to see if the mere laking of the corpuscles had anything to do with bringing about division, since the tap-water or distilled water, of course, lakes the fishes' blood-corpuscles at once. 1 per cent. salt solution with $\frac{1}{2}$ per cent. ammonium nitrate caused no alteration in the trypanosomes, which lived for quite the normal length of time. In $\frac{1}{2}$ per cent. sodium phosphate, division of the trypanosomes took place in the case of a perch, but was much delayed. This was used in order to see if any alteration in the behaviour of the flagellates was to be observed in the presence of the phosphorus, as this substance seems to influence the production of microgametes in Saprolegnia, KLEBS (8). Extract of medicinal leech made with distilled water brought about division, whereas extract made with 0·85 per cent. salt solution produced no division. Eel-serum was used to see if it caused any laking of fish blood, and also to find out if it in any way affected the trypanosomes. The blood-corpuscles showed only the very slightest signs of laking, and the parasites seemed utterly unaffected and lived for quite the usual length of time.

In these experiments, the infected blood, which when diluted with water showed the divisions and which when quite pure or when treated with such solutions as

1 per cent. NaCl, etc., showed no alteration, was often drawn from an individual fish on the same day and at the same time, so that the possibility of having struck a chance outburst of natural division of the parasite in the fish may be discounted. Such outbursts were never found at any time. Great caution is required in analysing the results of experiments where such complicated factors are involved; the one constant feature, however, in the cases above mentioned is that, where the salt-content of the blood was lowered by the fluid added, division of the trypanosomes occurred, and not under other conditions.

The osmotic pressure of the blood is about 7 atmospheres, that is to say, it is isotonic with a salt solution of 0·936 per cent. This estimation is that given by HAMBURGER (4) for the blood of the tench, and by HOEBER (6) for the blood of the barbel. The lowering of the osmotic pressure of the blood and the probable absorption of water by the trypanosome consequent upon this seems to be the stimulus which sets off the divisions. These divisions in the diluted blood are identical with the first divisions in the crop of the leech, and it is highly probable that the lowering of the osmotic pressure of the fluid in which the trypanosomes find themselves is at least one of the factors at work in bringing about the extraordinary burst of multiplication in the intermediate host. It may be said that by adding the water I have simply started a cultivation similar to what might occur in a blood-agar tube. That is doubtless the case. The only point of the experiments in question is that they give an indication of at least one, and probably one of the chief factors conditioning the said cultivation.

I have come across divisions of this kind on slides of blood with *T. vittatæ* from the milk-tortoise and with *T. raiæ* from the skate. In both cases I was in the habit of taking blood from the peripheral circulation without killing the animal, and have now no doubt that the blood must have been contaminated. Prof. MINCHIN and Dr. WOODCOCK, working with an excellent infection of *T. raiæ* at Rovigno, found no multiplication to occur in pure blood drawn from the heart. I hope to repeat these experiments with *T. raiæ* when opportunity offers.

This question of the absorption of water by the trypanosome is of course a similar phenomenon to that observed by LOEB (10) and other workers in the artificial parthenogenesis of sea-urchins' eggs. It is possible that the very stimulating change from the blood of the fish to the alimentary canal of the leech has taken the place of conjugation. The possibility of a chemical stimulus taking the place of fertilisation is clearly demonstrated in LOEB'S work, and there is no good reason why such a process should not occur in nature, more especially with such a relatively primitive organism as a flagellate. The point is at present one of pure speculation, but might yield some result when more work has been done along this line.

The whole life-cycle of the trypanosomes seems rather to support KLEBS' general theory (18) that the successive stages in the development of an organism are not determined primarily from within, but are due to internal changes brought about by

changes in the environment. For instance, the burst of division and consequent change of form does not take place until the parasite enters the leech ; so also the proboscis-form appears in response to certain digestive conditions and does not appear until those conditions are present.

A table is added of the various experiments dealing with the adding of reagents to the blood and their result. I wish to point out that fishes P and Q were newly infected with bream-trypanosomes.

Date.	Fish.	Reagent.	Result.
1910.			
June 30	Goldfish	Boiled distilled water	Trypanosomes divided.
July 7	Goldfish	Pure blood	Trypanosomes did not divide.
„ 20	Goldfish	Tap-water	Trypanosomes divided.
„ 22	Goldfish	Distilled water	„ „
August 4	Goldfish	Tap-water	„ „
„ 11	Goldfish	Extract of Hirudo made with *aq. dest.*	„ „
„ 12	Perch (1)	Tap-water	„ „
„ 12	Perch (1)	Extract of Hirudo made with *aq. dest.*	„ „
„ 13	Perch (1)	Extract of Hirudo made with 0·85 per cent. NaCl	Trypanosomes did not divide.
„ 15	Goldfish (18)	Tap-water	„ „
„ 15	Goldfish (18)	1 per cent. NaCl	„ „
„ 15	Goldfish (18)	Extract of Hirudo made with *aq. dest.*	„ „
„ 29	Goldfish (19)	Tap-water	„ „
„ 30	Goldfish (20)	Tap-water	Trypanosomes divided.
September 2	Perch (2)	Tap-water	„ „
„ 2	Perch (2)	1 per cent. NaCl	Trypanosomes did not divide.
„ 15	Goldfish (O)	1 per cent. NaCl	„ „
„ 15	Goldfish (O)	1 per cent. NaCl + $\frac{1}{2}$ per cent. ammonium nitrate	„ „
„ 15	Goldfish (O)	1 per cent. KCl	„ „
„ 17	Fish (Q)	1 per cent. acetic acid	Trypanosomes died at once.
„ 17	Fish (Q)	$\frac{1}{2}$ per cent. sodium phosphate	Trypanosomes did not divide.
„ 17	Fish (Q)	Pure blood	„ „
„ 24	Perch (7)	1 drop acetic acid to 5 c.c. *aq. dest.*	Trypanosomes died at once.
October 11	Fish (O)	Tap-water	Trypanosomes divided.
„ 20	Fish (Q)	Distilled water	A very few of the trypanosomes divided.
„ 20	Fish (Q)	Pure blood	Trypanosomes did not divide.
November 4	Fish (P)	Tap-water	„ „
„ 4	Perch (6)	Pure blood	„ „
„ 4	Perch (6)	Distilled water	Trypanosomes divided.
„ 4	Perch (6)	$\frac{1}{2}$ per cent. sodium phosphate	Trypanosomes divided, but division much delayed.
„ 17	Perch (7)	Tap-water	Trypanosomes divided.
„ 17	Perch (7)	Eel serum	Trypanosomes did not divide.
„ 18	Pike (1)	Tap-water	Trypanosomes divided.
„ 18	Pike (1)	Eel serum	Trypanosomes did not divide.
„ 18	Pike (1)	1 per cent. salt solution	„ „
„ 18	Pike (1)	1 per cent. ammonium nitrate	„ „
„ 18	Pike (1)	Pure blood	„ „
December 7	Perch (1)	1 per cent. potassium chloride	„ „

Summary.

(1) Trypanoplasms and trypanosomes are not transmitted from parent to offspring in the leech *Hemiclepsis marginata.*

(2) The trypanoplasm of the goldfish is transmitted from fish to fish by *Hemiclepsis marginata.*

(3) The trypanoplasms taken into the crop with the blood multiply and produce slender forms, which pass forward into the proboscis-sheath, from whence they are injected into the fish. A clean fish may show trypanoplasms in the blood examined microscopically on the fourth day after inoculation by means of an infected leech.

(4) Trypanosomes derived from goldfish in the Queensberry Lodge pond, from perch and bream from the Elstree Reservoir and from rudd from Histon in Cambridgeshire, all complete their cycle of evolution in *Hemiclepsis marginata.*

(5) The trypanosomes taken into the crop of the leech with the blood multiply very rapidly, undergoing a marked change of form (figs. 7–27). After some days slender forms begin to arise. These increase in number, and at the end of digestion, some time after the blood has quite disappeared, they come forward and lie in the proboscis-sheath. The form found in the sheath is a very slender long creature of quite a definite type; division has never been observed in this phase. When the leech feeds once more these individuals are inoculated into the fish. The proboscis-sheath is always cleared of trypanosomes by one feed. After a clean feed the slender trypanosomes disappear from the crop of an infected leech, and the infection is carried on by short broad forms. Conjugation has never been observed. The following passages have been made by means of clean leeches :—Goldfish to goldfish, perch to goldfish, bream to goldfish.

(6) If water is added to the blood of fish containing trypanosomes, the flagellates divide after a number of hours (six to nine hours), probably in response to the lowering of the osmotic pressure of the fluid in which they find themselves.

LIST OF PAPERS REFERRED TO.

1. BRUMPT, E. "De l'Hérédité des Infections à Trypanosomes et à Trypanoplasmes chez les Hôtes Intermédiaires," 'C. R. Soc. Biol.,' vol. 63, p. 176.
2. DOFLEIN, F. "Studien zur Naturgeschichte der Protozoen," 'Arch. f. Prot.,' vol. 19, 1910.
3. FRIEDRICH, —. "*Trypanoplasma helicis*," *ibid.*, vol. 19, 1910.
4. HAMBURGER, —. 'Osmotischer Druck und Ionenlehre,' vol. 1, p. 182, 1902.
5. HARDING, W. A. "A Revision of the British Leeches," 'Parasitology,' vol. 3, No. 2, July, 1910.
6. HOEBER, R. "Weitere Mittheilung über Ionenpermeabilität bei Blutkörperchen," 'Pflüger's Arch.,' vol. 102, 1904.
7. KEYSSELITZ, G. "Generations- und Wirtswechsel von Trypanoplasma borreli," 'Arch. f. Prot.,' vol. 7, 1906.
8. KLEBS, G. Croonian Lecture, 1910, 'Proc. Roy. Soc.,' B, vol. 82.
9. LÉGER, L. "Sur la Structure et la Mode de Multiplication des Flagellés du genre Herpetomonas, Kent," 'Compt. Rend. Acad. Sci.,' Paris, vol. 134, No. 14, p. 781.
10. LOEB, J. 'Untersuchungen über künstliche Parthenogenese,' 1906.
11. LUHE, M. 'Mense's Handbuch der Tropenkrankheiten,' vol. 3, 1906.
12. MINCHIN, E. A. "Observations on the Flagellates Parasitic in the Blood of Freshwater Fishes," 'Proc. Zool. Soc.,' 1909.
13. MINCHIN, E. A., and WOODCOCK, H. M. "Observations on certain Blood-parasites of Fishes occurring at Rovigno," 'Quart. Journ. Micro. Sci.,' vol. 55, p. 113, 1910.
14. PETERS, A. W. "Metabolism and Division in Protozoa," 'Proc. Amer. Acad.,' vol. 39, 1904.
15. PETRIE, G. F. "Observations relating to the Structure and Geographical Distribution of certain Trypanosomes," 'Journ. Hygiene,' vol. 5, 1905.
16. PIERANTONI, U. "Struttura, Biologia e Sistematica di *Anoplophrya paranaidis*, n. sp.," 'Arch. f. Prot.,' vol. 16, p. 82.
17. PLEHN, M. "*Trypanoplasma cyprini*, n. sp.," 'Arch. f. Prot.,' vol. 3.
18. PORTER, A. "The Life-Cycle of *Herpetomonas jaculum*, Léger, Parasitic in the Alimentary Tract of *Nepa cinerea*," 'Parasitology,' vol. 2, No. 4, 1910.
19. SCHUBERG, A., and KUNZE, W. "Uber eine Coccidienart aus dem Hoden von *Herpobdella atomaria—Orcheobius herpobdellæ*," 'Verh. Deutsch. Zool. Gesell.,' 1906.
20. THOMSON, J. D. "Cultivation of the Trypanosome found in the Blood of Goldfish," 'Journ. Hygiene,' vol. 8, p. 75, 1908.

DESCRIPTION OF FIGURES.

Plate 1.

The figures are drawn from stained specimens with the aid of Abbé's camera lucida; the magnification is 4000 linear.

Fig. 1. Trypanoplasm from the blood of the goldfish.

Fig. 2. Trypanoplasm of the goldfish from the crop of a young Hemiclepsis 44 hours after beginning to feed.

Fig. 3. Trypanoplasm from the crop of a young Hemiclepsis $3\frac{1}{2}$ days after feeding.

Fig. 4. Slender trypanoplasm from the crop of a young Hemiclepsis on 6th day after feeding.

Fig. 5. Trypanoplasm from crop of a young Hemiclepsis on 6th day after feeding.

Fig. 5a. Trypanoplasm from the crop of young Hemiclepsis on 7th day after feeding.

Fig. 6. Slender trypanoplasm from the proboscis-sheath of a young Hemiclepsis on the 10th day after feeding.

Fig. 6a. Trypanoplasm from the crop of a young Hemiclepsis on 25th day after feeding.

Fig. 7. Trypanosome from the perch newly ingested by a young Hemiclepsis, and still quite unaltered.

Fig. 8. Trypanosome showing earliest change in the crop of Hemiclepsis which had been feeding for $3\frac{1}{2}$ hours.

Fig. 9. Trypanosome from crop of young Hemiclepsis which had been feeding for $8\frac{1}{2}$ hours.

Figs. 10–13. Developmental phases from the crop of a young Hemiclepsis on 8th day after feeding.

Fig. 14. Early trypaniform phase.

Fig. 15. Long, somewhat slender form from the crop of a young Hemiclepsis on the 10th day after feeding.

Plate 2.

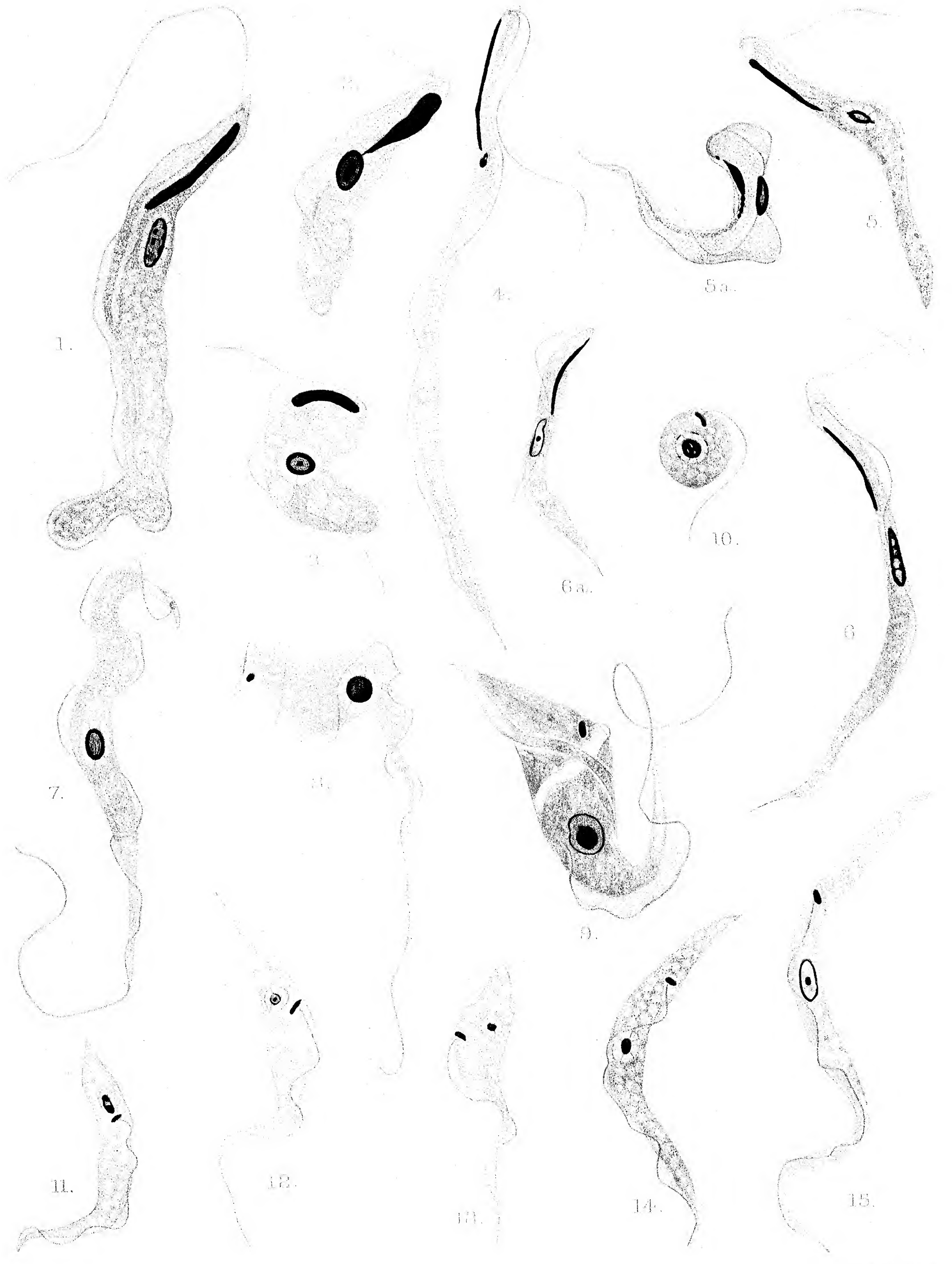
Fig. 16. Slender form from the crop of a young Hemiclepsis on 9th day after feeding.

Figs. 17 and 18. Slender inoculative type from the proboscis of an adult Hemiclepsis.

Figs. 19–21. Trypanosomes from the crop of a young Hemiclepsis well infected with trypanosomes from the bream, 24 hours after a feed of clean blood.

Fig. 22. Trypanosome from the crop of a young Hemiclepsis, well infected with trypanosomes from the bream, 5 days after a feed of clean blood.

Figs. 23–27. Division-stages from crop of the same leech as in fig. 22.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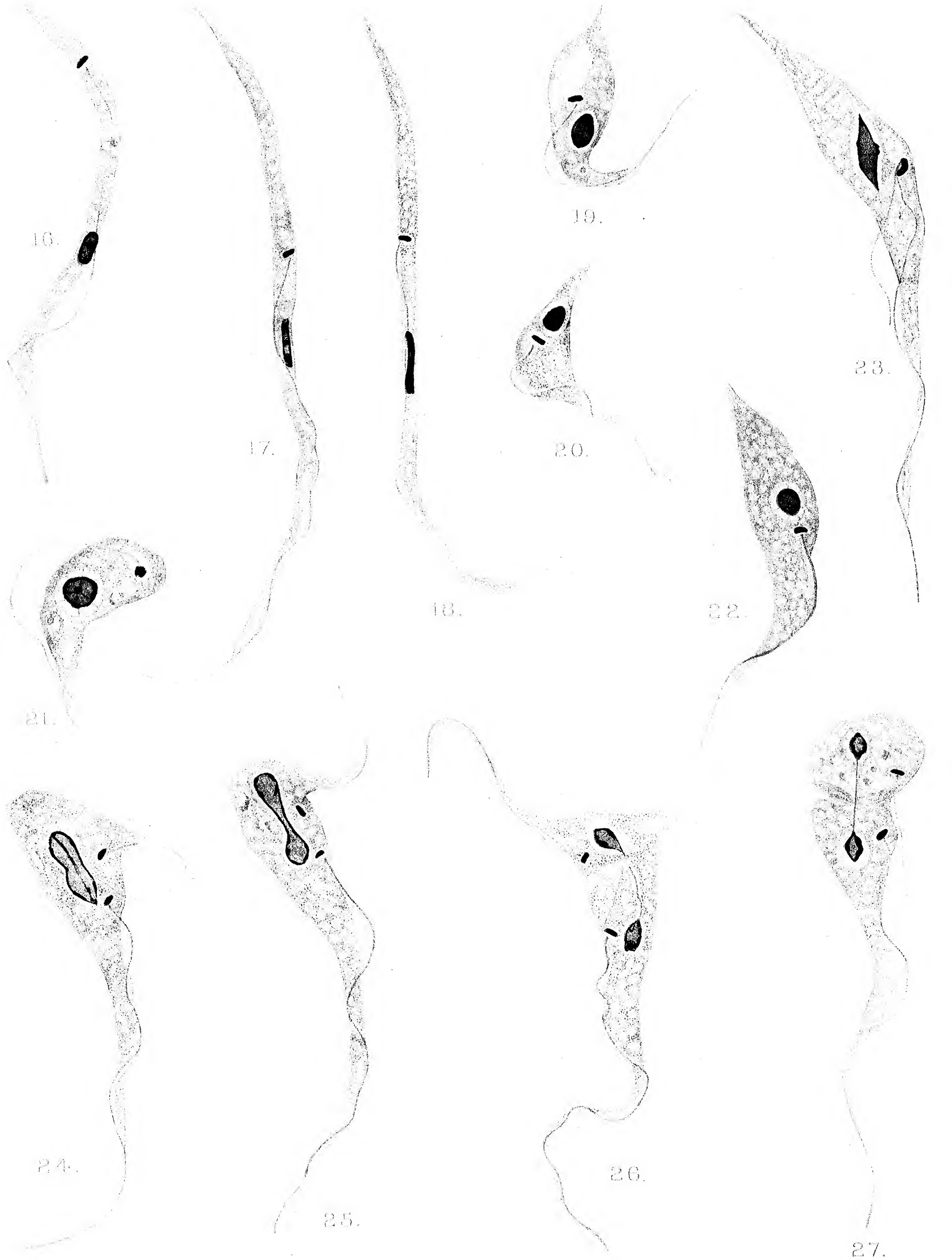

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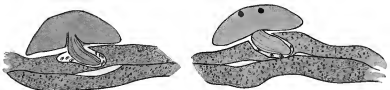
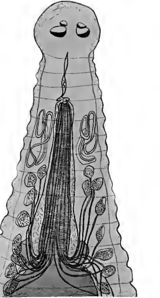


TEXT-FIG. 1.—Two Consecutive Sections of the Head of a Leech in the act of Sucking Blood from the Tail of a Goldfish.



TEXT-FIG. 3.—Anterior end of *H. marginata*, drawn from transparent living specimen. The flagellates may be seen as very minute threads in the proboscis-sheath.

PLATE 1.

The figures are drawn from stained specimens with the aid of Abbé's camera lucida; the magnification is 4000 linear.

Fig. 1. Trypanoplasm from the blood of the goldfish.

Fig. 2. Trypanoplasm of the goldfish from the crop of a young Hemiclepsis 44 hours after beginning to feed.

Fig. 3. Trypanoplasm from the crop of a young Hemiclepsis $3\frac{1}{2}$ days after feeding.

Fig. 4. Slender trypanoplasm from the crop of a young Hemiclepsis on 6th day after feeding.

Fig. 5. Trypanoplasm from crop of a young Hemiclepsis on 6th day after feeding.

Fig. 5A. Trypanoplasm from the crop of young Hemiclepsis on 7th day after feeding.

Fig. 6. Slender trypanoplasm from the proboscis-sheath of a young Hemiclepsis on the 10th day after feeding.

Fig. 6A. Trypanoplasm from the crop of a young Hemiclepsis on 25th day after feeding.

Fig. 7. Trypanosome from the perch newly ingested by a young Hemiclepsis, and still quite unaltered.

Fig. 8. Trypanosome showing earliest change in the crop of Hemiclepsis which had been feeding for $3\frac{1}{2}$ hours.

Fig. 9. Trypanosome from crop of young Hemiclepsis which had been feeding for $8\frac{1}{2}$ hours.

Figs. 10–13. Developmental phases from the crop of a young Hemiclepsis on 8th day after feeding.

Fig. 14. Early trypaniform phase.

Fig. 15. Long, somewhat slender form from the crop of a young Hemiclepsis on the 10th day after feeding.

PLATE 2.

Fig. 16. Slender form from the crop of a young Hemiclepsis on 9th day after feeding.

Figs. 17 and 18. Slender inoculative type from the proboscis of an adult Hemiclepsis.

Figs. 19–21. Trypanosomes from the crop of a young Hemiclepsis well infected with trypanosomes from the bream, 24 hours after a feed of clean blood.

Fig. 22. Trypanosome from the crop of a young Hemiclepsis, well infected with trypanosomes from the bream, 5 days after a feed of clean blood.

Figs. 23–27. Division-stages from crop of the same leech as in fig. 22.